2. Parasite Biology

MALARIA PARASITE BANK

Parasite Bank is supporting a large number of organisations working on various aspects of malaria. Biological materials including nonhuman and human plasmodia preserved / maintained at the Malaria Parasite Bank were supplied to various research organisations. The nonhuman parasites, especially *Plasmodium berghei* (both chloroquine resistant and sensitive) are being used for the *in vivo* screening of extracts / fractions of medicinal plants. During 2003 *P. falciparum* isolates adapted to *in vitro* culture conditions and characterised for drug susceptibility to different antimalarials have been supplied to scientists / researchers from Institutes / Universities for collaborative studies. A total of 48 *P. falciparum* isolates were supplied for studies on genetic variation of T-cell epitopes and another 20 isolates were supplied to IISc, Bangalore for molecular characterisation (Pfcrt). Few of the isolates characterised for their CQ sensitivity status, erythrocyte invasion properties, etc. were again cultivated *in vitro* to expand the number of vials cryopreserved at the Parasite Bank for future use / supply.

P. falciparum isolates from Goa, Maharashtra, Car Nicobar, Gautam Budh Nagar, Assam and Orissa showed high multiplicity of infection and prevalence of K1 and MAD20 of MSP-1, FC27 and 3D7 of MSP-2; R033 (< 25%) proportions.

One CQ resistant isolate has been cloned this year. Eleven clones were separated from this isolate and these clones were given for checking their monoclonality by molecular analysis. After expanding these clones were tested for CQ sensitivity. All the clones were found to be resistant to CQ in varying concentrations (8-32 p moles).

A study has been initiated to see the morphological changes taking place in cerebellar Purkinje cells and in the surrounding parenchymal cells during cerebral malaria. The study aims to see the effect of plant extracts with antimalarial properties in cerebral malaria in experimental animal model using Swiss albino mice and *P. berghei ANKA* strain. The initial studies showed that there is a reduction in the number Purkinje cells in *P. berghei* ANKA infected mice compared to uninfected normal animals.

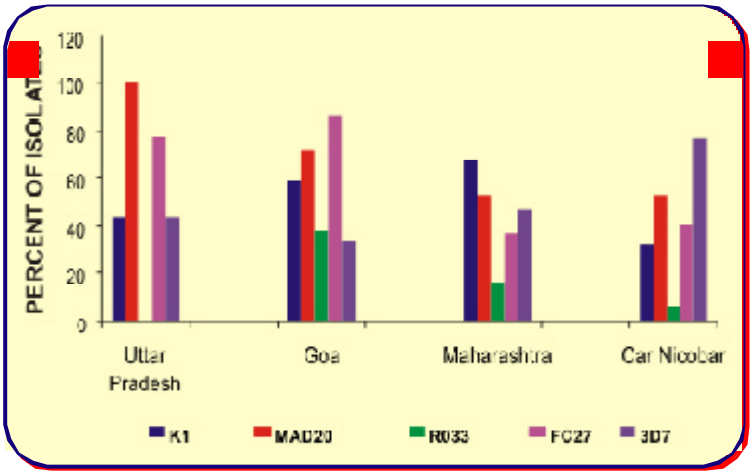
Characterisation of Human Malaria Parasites—*P. falciparum* and *P. vivax*

In continuation of earlier work, field isolates of *P. falciparum* collected from Goa, Maharashtra, Car Nicobar and Gautam Budh Nagar (U.P.) were analysed for polymorphism of MSP-1 and MSP-2, microsatellite markers and *P. vivax* from Rourkela and Goa were analysed for MSP-3α housekeeping gene (ribosomal protein L_{35}e) and SSU rRNA pattern. In addition, *P. falciparum* samples from the Parasite Bank were also analysed for MSP-1 and 2 polymorphism to check their clonal nature.

P. falciparum

MSP-1 and MSP-2: MSP-1 and 2 exhibited high degree of polymorphism among the isolates studied from different areas. In MSP-1, families K1 and MAD20 were prevalent in all the study areas, while R033 was present in proportions less than 25% except in Goa where it was observed in about 40% of the isolates. In MSP-2, both the families FC27 and 3D7 were prevalent in the study areas. High multiplicity of infection was observed among the isolates. Fig. 2.1 shows the areawise distribution of various families among the isolates.

Six out of 10 pairs of *P. falciparum* samples showed different genotypes of MSP-1 and MSP-2 on the day of recrudescence



Microsatellite markers: For the first time, microsatellite markers were used for analysis of the Indian *P. falciparum* isolates. Eleven microsatellite markers namely TA1, TA60, Polyα, Ara2, PfPg 377, 2490, TAA81, TAA109, TAA87, TAA42 and PfPK 2 were used. Analysis revealed highly polymorphic nature of Indian isolates and number of alleles observed ranged from 4 to 10.

Microsatellite markers have been used for the first time for the analysis of Indian *P. falciparum* isolates. Field isolates analysed are found to be highly polymorphic and number of alleles observed were minimum of four and maximum of ten

Parasite Bank isolates: Samples received from the parasite bank were genotyped for MSP-1 and MSP-2 using family specific nested PCR assays. All the samples were observed to be monoclonal in nature (Fig. 2.2).

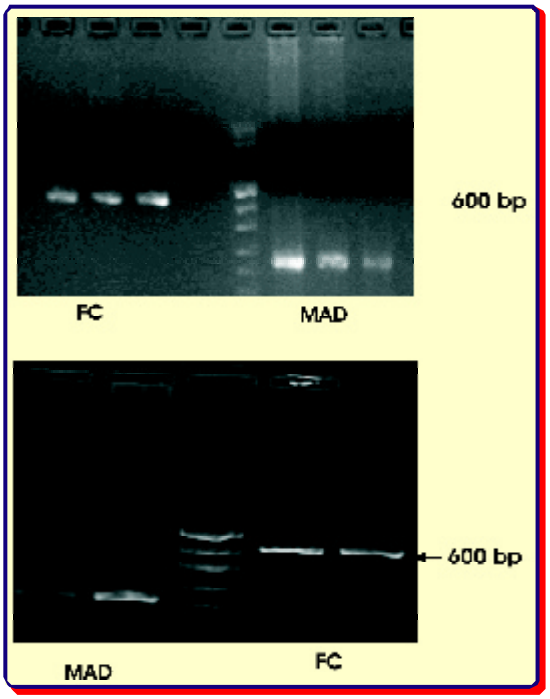


Fig. 2.2: Electrophoretogram showing MSP-1 and MSP-2 profiles of samples received from malaria parasite bank

Purpose of this was to select few pure clones which can be used in further research.

P. vivax

MSP-3α: Isolates from, Goa and Orissa were analysed using PCR-RFLP. Nested PCR revealed three common variants of sizes approximately 1.2, 1.4 & 1.8 kb with 1.8 kb variant being the most common. RFLP pattern with Alu I and Hha I revealed highly polymorphic nature of the isolates. During the study it was observed that H 1 pattern of Hha I digestion was observed frequently in 1.2 and 1.4 kb variants, while it was totally absent in the most common 1.8 kb variant. Therefore, in future efforts will be made to sequence this variant.

SSUrRNA: Geographically distributed population of *P. vivax* reveals that there are two separate sub populations of *P. vivax*. The populations of *P. vivax* from America and European countries form a group distinct from those of Asia and Africa. Parasites inhabiting in America and Europe are designated as new world isolates and those in Asian and African countries are designated as old world isolates. Comparing the 18S rRNA sequences of the new and old world isolates, a consistent polymorphism that separates the two according to geographic location was observed. Preliminary study carried out by MRC has shown the presence of both the forms— new and old world type in Indian isolates (Fig. 2.3). Sequence data has confirmed the two types in limited number of isolates.

SSU rRNA analysis of *P. vivax* field isolates revealed that in India both types of *P. vivax*, i.e. old world (Asian/ African) and new world (European/ American) isolates were found

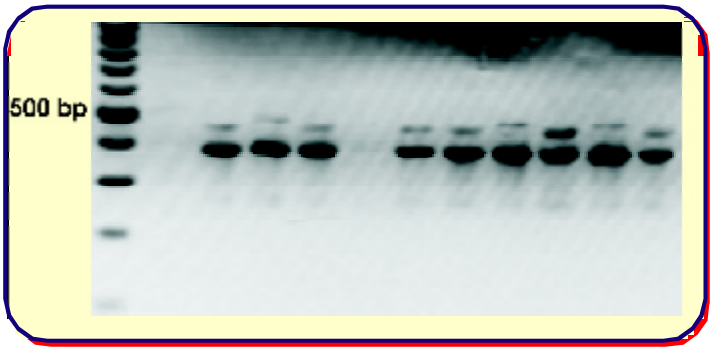


Fig. 2.3: Electrophoretogram showing SSU rRNA analysis of Indian *P. vivax* isolates

Genetic Diversity of *P. falciparum* and *P. vivax* in India using Molecular Markers (ICMR Funded Project)

Surveys were conducted for malaria prevalence in Sonapur district (Assam), Sundargarh district (Orissa) and Chennai (Tamil Nadu) to collect field isolates. In Delhi, patients attending the malaria clinic of Malaria Research Centre at Nanak Enclave campus were enrolled for the study. *P. falciparum* and *P. vivax* isolates have been collected from patients diagnosed positive for malaria by microscopy.

Field isolates of *P. falciparum* have been collected from Sundargarh district (Orissa) and Sonapur district (Assam) and field isolates of *P. vivax* from Delhi and Chennai (Tamil Nadu). Follow-up of the patients is being carried out to collect samples with primary and repeated episodes (recrudescence in *P. falciparum*

and relapse in *P. vivax*). A total of 18 paired samples of *P. falciparum* and 21 of *P. vivax* have been collected.

P. falciparum

Analysis of MSP-1 and MSP-2: A total of about 48 field isolates collected from Assam and Orissa have been analysed for polymorphism of MSP-1 (block 2) and MSP-2 (block 3). Results revealed polymorphism of both the systems among the isolates of both the areas.

Twenty-two isolates of *P. falciparum* from Assam have been analysed for family grouping and observations revealed the presence of all the three families of MSP-1 (K1, MAD20 and RO33) and both of MSP-2 (FC27 and 3D7). Proportional prevalence of family specific markers is 72.7% each for K1 and MAD20; 22.7% for RO33 of MSP-1 and 40.9% for FC27 and 81.8% for 3D7 of MSP-2. Out of twenty-two samples analysed 91% were multiclonal. Only two isolates were categorised as single clonal based on the genotyping of MSP-1 and 2.

Twenty-six isolates of *P. falciparum* from Sundargarh district (Orissa) have been analysed for MSP-1 and MSP-2 family grouping. All the three families of MSP-1 and two of MSP-2 were observed with a prevalence of 73% for K1, 38.5% for MAD20, 22.6% for RO33 of MSP-1 and 65.4% for FC27 and 96.1% for 3D7 of MSP-2. About 96% of the isolates were observed multiclonal based on genotypes of both MSP-1 and MSP-2.

Allelic polymorphism of MSP-1 and 2 observed is slightly more among Orissa isolates compared to that of Assam isolates, however, allele sizes observed were almost similar in both the areas. As observed in earlier studies, RO33 is monomorphic in both the areas.

Analysis of microsatellite markers: Thirty isolates from Sundargarh district, Orissa were analysed for eleven microsatellite markers using multiplex PCR and genotyping. Results revealed that more than 90% *P. falciparum* field isolates from Sundargarh district comprised of multiple infection of genetically different genotypes. All the eleven markers studied were observed to be highly polymorphic among Indian isolates.

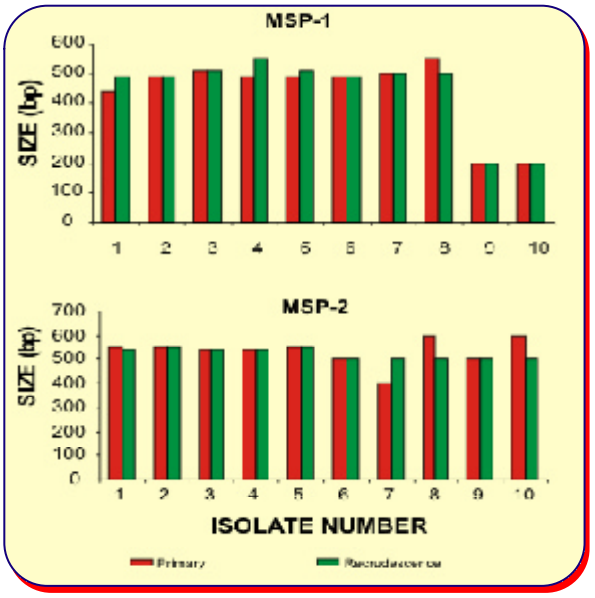


Fig. 2.4: Graphs showing the analysis of primary infection and recrudescence in *P. falciparum* isolates

Analaysis of recrudescence infection: A total of ten paired samples collected from *P. falciparum* positive patients were analysed for genotyping of MSP-1 and MSP-2. Results revealed that out of 10 pairs of samples analysed six showed different genotypes on the day of recrudescence. Fig. 2.4 shows the genotype analysis of *P. falciparum* samples collected on Day 0 and the day of recrudescence.

P. vivax

Analysis of MSP-3α: Isolates from Delhi and Chennai were analysed using PCR-RFLP. Nested PCR revealed three common variants of sizes approximately 1.2, 1.4 and 1.8 kb, of which 1.8 kb variant is common with frequency of occurrence being above 60%. A rare variant of 0.5 kb was also observed in one of the Chennai isolates. RFLP pattern with Alu I and Hha I revealed highly polymorphic nature of the isolates. During the study it is observed that H1 pattern of Hha I digestion was observed frequently in 1.2 and 1.4 kb variant, while it is totally absent in 1.8 kb variant and was observed in both the areas.

Therefore, in future efforts will be made to sequence samples showing this pattern to confirm its association with 1.2 and 1.4 kb variants. Fig. 2.5 shows the PCR amplified size variants of MSP–3α and their distribution among the isolates.

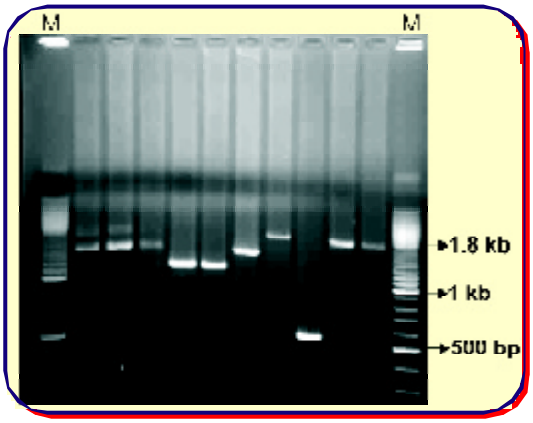


Fig. 2.5: Agarose gel electrophoretogram showing size variations in PCR amplified products of MSP-3α in *P. vivax* field isolates

A rare variant (approx. 0.5kb) of Pv MSP-3α was observed among Chennai field isolates of *P. vivax*. It is further observed that H1 genotype of Hha I digestion is associated with 1.2 & 1.4 kb variants

Rare variant (0.5 kb) observed in one of the Chennai isolates will also be sequenced to get structural details. Restriction digestion with Hha I revealed the absence of Hha I sites, while with Alu I revealed digested products.

Analysis of Ribosomal Protein L_{35}e: Isolates from Delhi and Chennai with single infection of *P. vivax*, ascertained by MSP-3α screening were amplified using specific primers and agarose gel electrophoresis revealed an amplified product of approximately 600 bp. Products were sequenced and analysed for single nucleotide polymorphism (SNP). SNPs observed were spread throughout the fragment length and not restricted to particular position. Fig. 2.6 shows the PCR amplified

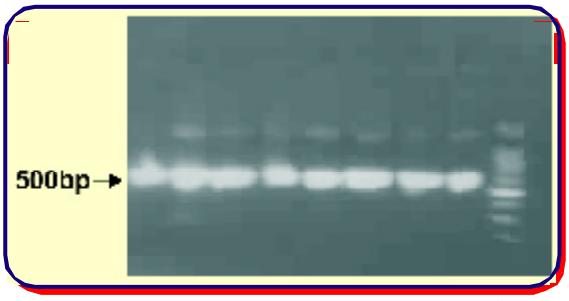


Fig. 2.6: Electrophoretogram showing PCR amplified product of L_{35}e (ribosomal protein) in Indian *P. vivax* field isolates

product in the isolates. Alignment of sequences of L_{35}e from field isolates of Delhi and Chennai showed a good alignment with SNPs.

Studies have been initiated on the sequence diversity of house keeping gene, L_{35}e and Duffy binding protein (DBP) of *P. vivax* field isolates

DBP : Sequences of duffy binding protein (DBP) from Papua New Guinea, Colombia and Korea available from NCBI were aligned and based on consensus sequences of region II, primers were designed covering all 12 cystein residues of region II, functional domain. Isolates from Delhi and Chennai were amplified using hemi-nested PCR and an amplified product of approx. 1.0 kb was obtained. Fig. 2.7 shows the amplified DBP gene from Indian isolates. PCR products were purified and cloned in pGEMT vector. The recombinants when sequenced showed absolute homology with those of Papua New Guinea except for few single nucleotide polymor-phisms.

Fig. 2.7: Electrophoretogram showing PCR amplified product of DBP in Indian *P. vivax* field isolates

Studies on Genetic Polymorphism of T-cell Epitopic Region of Circumsporozoite Protein (CSP) of *P. falciparum* from India—Relevance for Vaccine Development (a CSIR Sponsored Project)

Expression studies of different prototype variants of T-cell epitopic regions of Pf CSP have been initiated

The importance of T-cells in malaria immunity has been appreciated for a long time. However, T-cell epitopes show variation. Two T-helper cell epitopes (Th 2R and Th 3R) have been identified in CSP. Genetic variation in Th-epitopes of CSP in *P. falciparum* was studied in 54 isolates collected from subjects from different regions of India—Delhi, Uttar Pradesh, Assam, Rajasthan and Orissa. The T-cell epitopic region was amplified by polymerase chain reaction (PCR) using primers corresponding to nucleotides 1008–1028 and 1323–1347 in the CSP gene sequence of 7G8 clone. The amplified products after purification were sequenced using the same primers which are used to amplify the T-helper cell epitopic regions.

An apparent trend of regionally unbiased restricted polymorphism was observed . The variations can be grouped. Therefore, different prototype variants from the groups could be included in a sub-unit polyvalent vaccine againt sporozoite. However, further studies are needed to establish this observation.

The amplified T-helper cell epitopic regions were cloned into pQE-40 vectors (QUIAGEN) at the Sma I site. Competent *E. coli* M15 strain (QUIAGEN) were transformed both with pQE-40 and pQE-40 I (pQE vector with insert). Positive clones were characterised by PCR using the same primers used to amplify the T-

helper cell epitopic regions. Expression of the T-helper cell epitopic regions is in progress.

Reactivity of Monoclonal Antibodies with *P. vivax* Infected Erythrocytes and Parasite Lysate

Ten hybridoma lines isolated earlier were grown in large volume. Culture supernatants were checked by the ELISA and IFA tests with *P. vivax* and *P. falciparum* crude lysates and erythrocyte smears. All ten antibodies showed reactivity with *P. vivax* erythrocytic stages in immunofluorescence assay (Figs. 2.8 a–d). Immunoglobulins were fractionated from an individual batch by ammonium sulphate precipitation. They were then passed through Protein-A sepharose for obtaining pure fractions. Supernatant of three clones were tested for their reactivity with *P. vivax* lysate. On western blot, these antibodies reacted with 40–42 kDa proteins of a pooled preparation of *P. vivax* parasites collected from different geographic areas of the country (Fig. 2.9). The affinity-purified fractions isolated from three different clones were coupled with 6MB-sepharose for the isolation of specific proteins from *P. vivax* parasitised erythrocyte lysate by affinity adsorption. Purified fractions were checked for reactivity with respective monoclonal antibody (Fig. 2.10). Proteins were then transferred onto

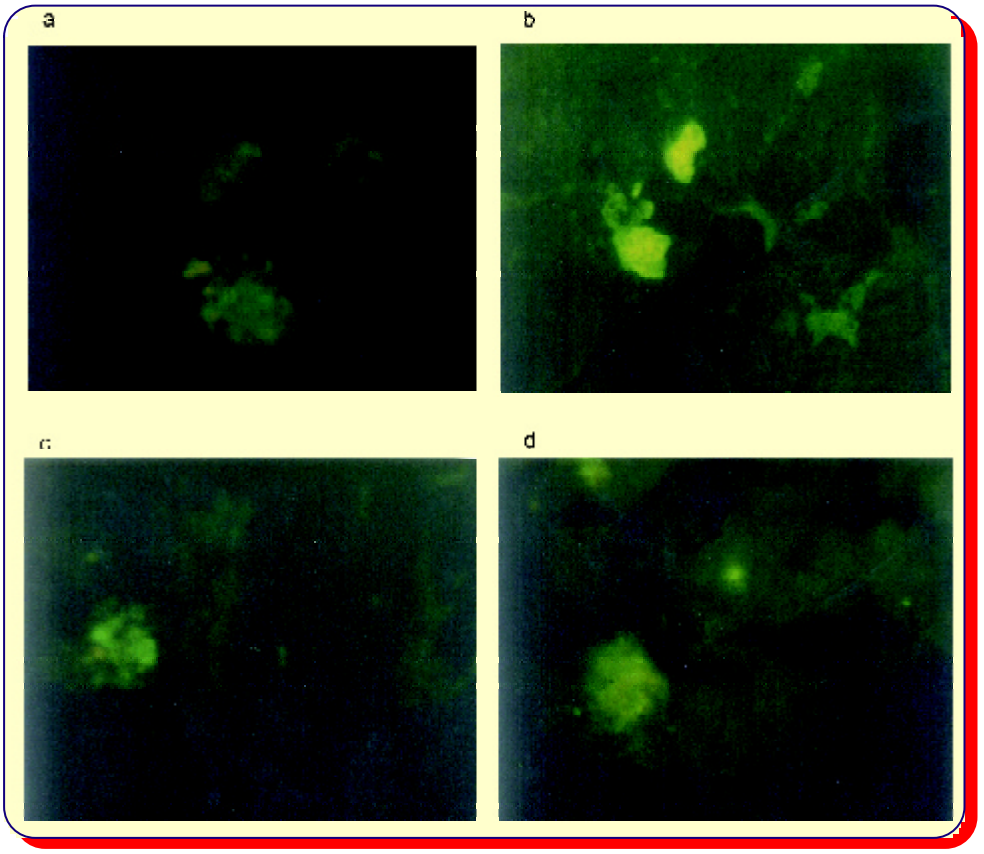


Fig. 2.8 (a-d): Reactivity of monoclonal antibodies with *P. vivax* in IFA

PVDF membrane and N-terminal sequencing was performed. The sequence homology search was done on available database. The protein sequences showed 45–75% homology with the family of *vir*-proteins of *P. vivax*.

Ten hybridoma lines showing reactivity with *P. vivax* erythrocytic stages in immunofluo-rescence assay and western blots are available

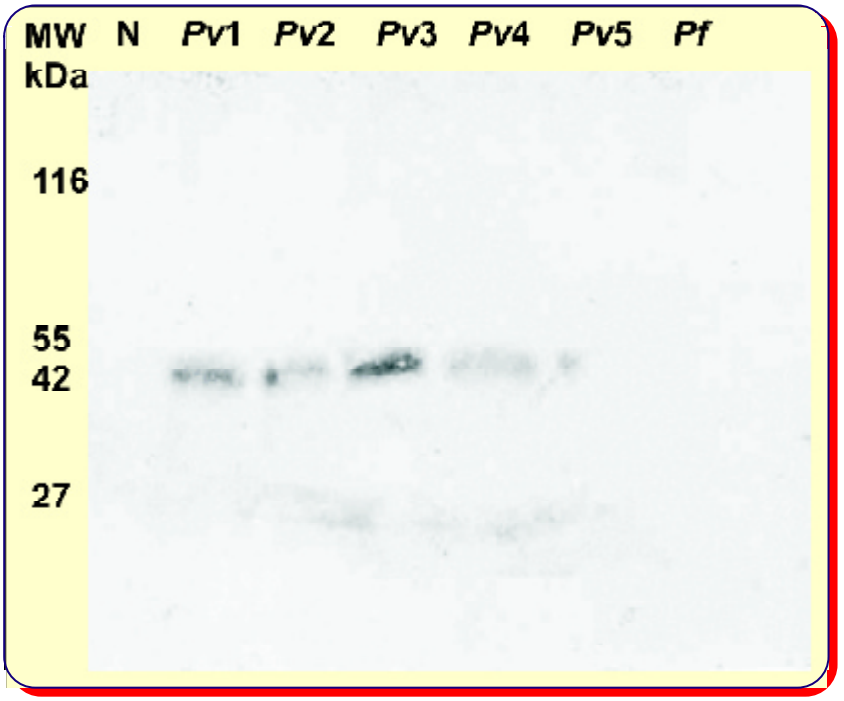


Fig. 2.9: Western blot of *P. vivax* lysates (*Pv*1–*Pv*5), *P. falciparum* lysate (*Pf*) and normal human erythrocyte lysate (N). A protein of ~40–42 kDa reacted with monoclonal antibody (MAb.1)

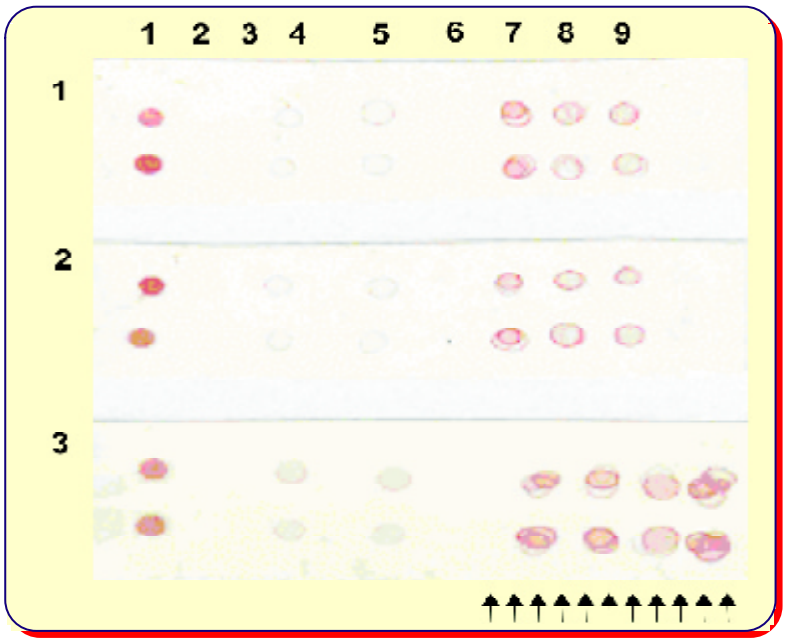


Fig. 2.10: Reactivity of purified proteins from *P. vivax* lysate with three different monoclonal antibodies. 1–MAb (1:10); 2–Eluent; 3–PvAg (1:100); 4–*Pv* (unbound; 5–Eluent; and 6-9–Fractions

Comparison between Serum and Filter Paper Absorbed Blood to Determine Immunoglobulin Isotype Profile by ELISA

Present study was aimed to assess the reliability of the use of filter paper absorbed blood for the estimation of immunoglobulin G and M isotypes and inter-test comparison with corresponding serum samples. Antimalarial IgG and IgM were detected by enzyme immunoassay using finger-prick blood samples collected in capillary tubes and also spotted on Whatman filter paper. Assay was done in 92 blood samples obtained from 53 falciparum malaria patients, 23 fever cases (malaria negative) and 16 healthy individuals. A simple indirect ELISA was done using *P. falciparum* lysate and MSP-1_{19} peptide as antigens. Total IgG and IgM contents were also estimated in individual serum and filter paper (FP) elute by single radial immunodiffusion (SRID). Assay results of both serum and filter paper were compared (Figs. 2.11 and 2.12). The sensitivity and specificity of the assays for IgG measurement were comparable between serum and filter paper ($p<0.001$), whereas in case of IgM, detection level was poor in filter paper as observed by ELISA and SRID.

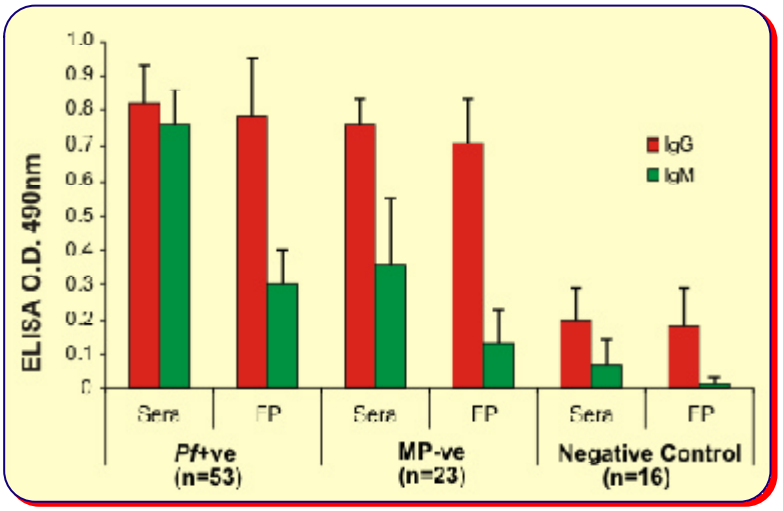


Fig. 2.11: Antimalarial IgG and IgM profiles in sera and *Pf* elutes against FP lysates

Filter paper blood spots could be used for determining IgG profile without any significant loss and also in seroepidemiology

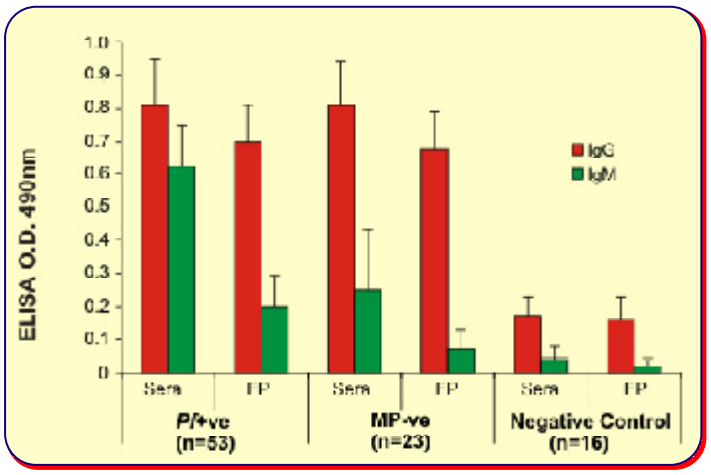


Fig. 2.12: Antimalarial IgG and IgM profiles in sera and FP elutes against MSP-1 peptide

For conducting serological surveys, field logistics are important for operational feasibility. Serological tests would be successful in producing relevant information in epidemiological surveys if the factors were well taken care of. Collection of blood samples and their transportation with proper storage from periphery to central facility carry a lot of importance in success of the test. Collection of finger-prick blood on filter paper from individuals of younger age group, especially children and infants is comparatively easier and acceptable than venipuncture. This advantage compensates for the slight loss in sensitivity when FP are titrated instead of sera. From the present study it may be concluded that filter papers to collect and handle blood samples are of great practical advantage in field studies. Blood samples absorbed on filter paper could be used for determining IgG profile without any significant loss in titre, and could also be applicable in seroepidemiological survey of other diseases. For determining primary or current or recent infection on the basis of IgM titre, serum samples are better than filter paper elutes.

Age-related Prevalence of Antibody Response against Five Defined *P. falciparum* Antigens

Age related prevalance of antibody response against Pf CSP, MSP-1, AMA-1 EBA-175 and Pfg 27 revealed an age-wise increasing pattern in antibody response

The occurrence of the humoral response to defined *P. falciparum* antigens was studied in 86 falciparum malaria patients from villages of Loni PHC, Ghaziabad. The antigens used for indirect ELISA were synthetic peptides derived from *Pf* circumsporozoite protein (CSP), merozoite surface protein (MSP-1), apical membrane antigen (AMA1), erythrocyte binding antigen (EBA175) and gametocyte antigen (PfG27).

Finger-prick blood samples were collected for sera from patients to conduct this test. Sera were tested for determining antimalarial IgG antibody against five stage-specific peptides by ELISA. An age-wise increasing pattern in antibody responses has been observed against all five antigens (Fig. 2.13). However, there was difference in the amount of antibody reaction as observed in ELISA O.D. values. The humoral response to these specific antigens occurred concurrently. Antibodies were detected in most of the adult sera compared to children. The

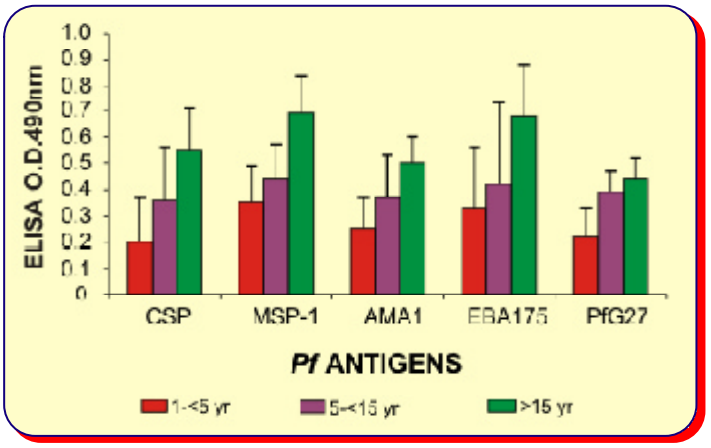


Fig. 2.13: Mean ELISA values for *P. falciparum* patients of different age groups

frequency of seropositivity increased with age approaching, but not reached adult levels by the age of 15 years.

Purification and Characterisation of a Haemoglobin Degrading Aspartic Protease from the Malarial Parasite *Plasmodium vivax*

Aspartic proteases of the human malaria parasite, *P. falciparum* are believed to play a key role in the essential pathways of merozoite release, invasion and haemoglobin degradation during the intraerythrocytic stages of its life cycle. Therefore, we have purified and characterised *P. vivax* aspartic protease to determine if this enzyme can be used as potential drug target and its inhibitors as potential antimalarial drugs. A *P. vivax* aspartic protease has been purified by a combination of ion exchange size exclusion chromato-graphy and HPLC. Its properties were examined to define their role in the haemoglobin degradation process. The purified enzyme migrated as a single band on the native PAGE and SDS / PAGE with a molecular mass of 40 kDa (Fig. 2.14). Gelatin zymogram analyses revealed a clear zone of proteolytic activity, which corresponded to the band obtained with native PAGE and also SDS / PAGE (Fig. 2.15 a & b). The enzyme had an optimal *pH* of 4.0 and exhibited its highest activity at 37°C. The enzyme was inhibited by pepstatin and IC_{50} value was found to be 3.5 mM, but not inhibited by other inhibitors namely *0*-phenanthroline, EDTA, PMSF or E-64

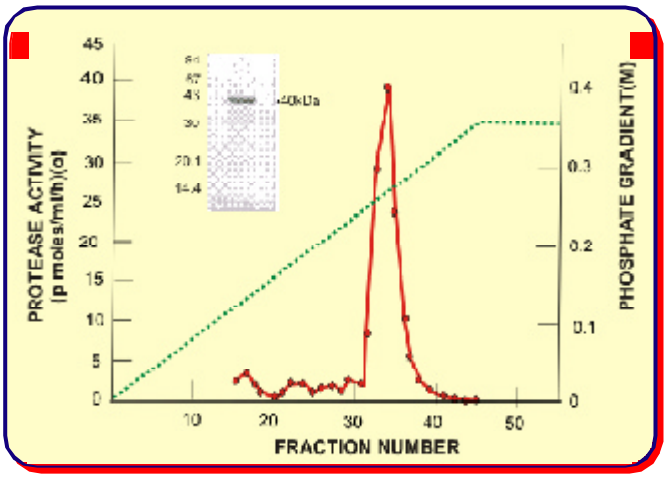


Fig. 2.14: HPLC purification of aspartic protease activity on a Biogel HPHT column using a Shimadzu 10A apparatus. The column was eluted with a sodium phosphate gradient, 0.01–0.35 M, at *pH* 6.8. The flow rate was 0.8 ml / min, and 0.8 ml fractions were collected and assayed for aspartic protease activity. *Inset* : SDS-PAGE analysis. A 50 µl aliquot of the HPLC peak was subjected to electrophoresis on a 12% SDS PAGE gel under reducing conditions. The gel was developed with Coomassie blue.

***P. vivax* aspartic protease has been purified and charecterised for field isolates**

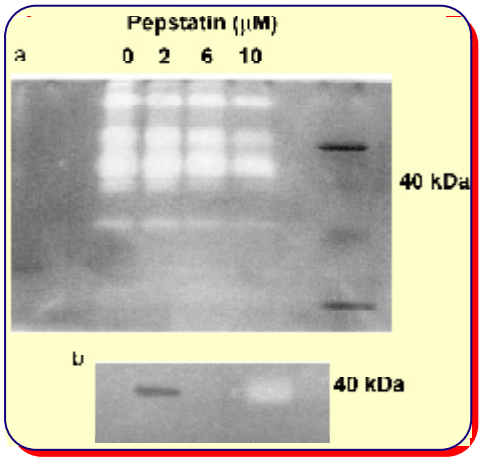


Fig. 2.15 (a): Zymogram (Gelatin gel PAGE) showing the aspartic protease activity (40 kDa) and its inhibition by different concentrations of pepstatin (2, 6, 10 µM); **(b):** SDS-PAGE and Zymogram (Gelatin gel PAGE) showing the purified aspartic protease protein and activity (40 kDa)

supporting its designation as aspartic protease. A lineweaver burk double reciprocal plot with pepstatin showed that the inhibition was competitive with respect to the substrate. Ca^{+2} and Mg^{+2} ions enhanced the protease activity whereas Cu^{+2} and Hg^{+2} ions were found to inhibit the enzyme activity. The pivotal role of aspartic protease inhibitors in development of / as new drugs in *P. vivax* may allow rational drug design for a new class of antimalarials. The results of this study have been compiled and ready for publication.

Parasite Killing in *Plasmodium vivax* Malaria by Nitric Oxide: Implication of Aspartic Protease Inhibition

In the present study, we examined the abilities of NO donors and NO producers to inhibit the plasmepsin activity in purified *P. vivax* extracts in a dose-dependent manner . The results provide new insights into the regulation of NO production in *P. vivax* malaria and also the mechanism of killing of malaria parasites via inhibition of protease activities, and may help us design novel strategies for selectively upregulating NO production for the inhibition of *P. vivax* malaria (Figs. 2.16 and 2.17).

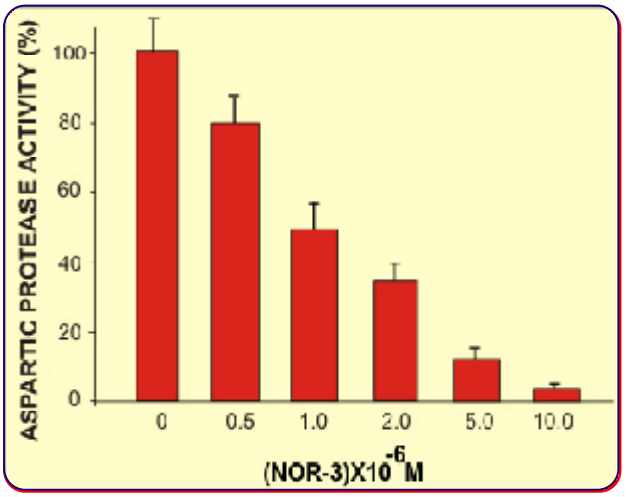


Fig. 2.16: Effect of NO donor NOR-3 on the activity of *P. vivax* aspartic protease and dose dependent inhibitory effect of NOR-3 on the enzyme activity.

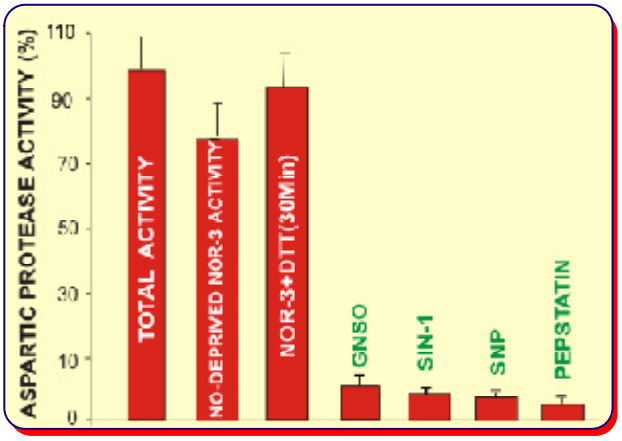


Fig. 2.17: Effect of NO on the catalytic activity of *P. vivax* aspartic protease. NO-deprived NOR-3 (NOR-3*; 1.0 x 10^{-5} M) does not affect the aspartic protease activity. The addition of DTT (1.0 x 10^{-3}M) to the inactive enzyme restores its activity. GNSO, SNP and SIN-1 block the aspartic protease activity. As a control, pepstatin, a typical aspartic protease inhibitor, suppresses the aspartic protease activity.

Glycophospholipid (GPL) as a Candidate Antigen for Diagnosing *Pf* Infection by Laser Immunoassay

GPL is very specific and sensitive to determine *Pf* positive case detection. Crude GPL was fractionated by thin layer chromatography, silica gel glass column chromatography. With increasing methanol concentrations it was possible to fractionate crude GPL into three fractions by column chromatography. Preparative thin layer chromatography also gave three bands. Each fraction showed the presence of docohexanoyl (lipid), sugars and phosphate. By mass spectrometry, three fractions (10, 50 and 70%) from methanol elute showed four core lipid components. Nuclear magnetic resonance spectroscopy was done in CDCL3 indicated 50 and 70% fractions have carbohydrate components. High performance liquid chromatography indicated the presence of six different sugar moieties. Glycophospholipid crude as well as pure fractions were tested against *P. falciparum*, *P. vivax* and control samples. Each component was highly specific and sensitive towards *Pf* infection. Antigenicity determined by ELISA technique showed poor specificity and sensitivity compared to that of LIA. Attempts are being made to develop matrix for enhancing binding with GPL antigen for large-scale immunodiagnosis purposes.

Screening of Medicinal Plants for their Antimalarial Property

Four out of 50 plant extracts tested from different parts of the country showed good antiplasmodial activity.

There is an urgent need for new antimalarials particularly to those parasites which are showing resistance to the existing antimalarials. The medicinal plants or parts of plants used for the treatment of fever in rural / tribal areas were collected from different geographical regions of India and tested for their antimalarial properties. A total of twenty-two medicinal plants were tested *in vitro*, for their antiplasmodial activity after preparing the crude ethanol extract. The *in vitro* test was done using both chloroquine sensitive and resistant isolates of *P. falciparum*. Schizont maturation inhibition (SMI) assay was used for the study. *In vivo* schizontocidal activity was done for one of the plant extracts, which was found effective in *in vitro* screening. Out of these, two extracts were further fractionated with chloroform, hexane, ethyl acetate and butanol. Chloroform fractions of these extracts were showing good activity. This material has been given for further processing by HPLC. Result is awaited.

Based on the results of the schizontocidal activity, one compound having four ingredients was prepared and schizontocidal assay was conducted *in vivo* using *P. berghei* and Swiss albino mice. Six groups, of five animals each, were taken for the study. All the animals were inoculated with *P. berghei* on Day 0. The drug in different doses (10, 30, 50 and 100 mg) were given to four experimental groups. The drug was administered orally for four consecutive days starting from Day 0. One group received no drug which served as control. The parasitaemia was monitored on Day 4. The inhibition percentage in the four experimental groups was 2.58, 52.9, 75.48 and 64.57% respectively. It is interesting to note that even though the group received 50 mg / kg body weight had an inhibition of

75.48% in parasitaemia on the Day 4, all the five animals survived for a very long time whereas the animals from the other groups died. Further studies are in progress.

Twenty extracts received from DRDO have been tested and out of these two were having very good antiplasmodial activity. Besides these, as a collaborative work, eight fractions of medicinal plants were tested *in vitro* for RMRC, Dibrugarh, Assam. One of these fractions was found to be having very good antiplasmodial activity.